TENASCIN AND WOUND HEALING IN THE HUMAN CORNEA.

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Purpose: Tenascin is an extracellular matrix glycoprotein which is expressed transiently in wound healing in human skin and animal corneas. *In vitro* and embryological studies suggest that tenascin mediates cell adhesion/anti-adhesion, enhancement/inhibition of cell proliferation, and promotion of cell migration. At present there are no published data regarding human corneal wound healing, therefore we have examined tenascin expression in corneas of histopathology specimens obtained because of previous trauma.
Method: 6μm sections were cut from globes with perforating corneal injury (20), blunt corneal injury (5) and 5 penetrating keratoplasty specimens in which there had been previous surgery. Cases were selected to encompass a range of time intervals between injury and removal (range <24 hours to 90 years). 10 globes enucleated for choroidal melanoma provided normal corneas. Tenascin was demonstrated with a monoclonal antibody specific for human tenascin (Dako TN2), revealed by an avidin-biotin peroxidase complex (ABC) technique.
Results: Strong TN2 immunopositivity was observed in regions of active inflammation, fibroblastic activity and vascularisation. Corneas from globes which had been enucleated within 7 days following injury were tenascin negative, while those which had remained *in situ* for over 7 days were positive. However, no tenascin was detected in mature, avascular scar tissue. Normal corneas were tenascin negative.
Conclusion: These results indicate that there is a temporal sequence of tenascin expression in human corneal wound healing. This, together with the expression of tenascin in areas of fibroblastic activity and vascularisation supports the view that tenascin is involved in human corneal wound healing processes.

A SIMPLE ORGAN CULTURE MODEL FOR CORNEAL WOUND HEALING STUDIES: THE EFFECT OF GROWTH FACTORS

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Purpose. To develop a simple organ culture model with which to study the mechanisms of corneal re-epithelialisation and the effect of growth factors.
Methods. Excisional trephine and epithelial scrape wounds were created on bovine and human corneo-scleral rings in which the endothelial corneal concavity was then filled with an agar/collagen mixture. Organ culture was undertaken at 37°C in a humidified 5% CO_2 incubator with serum-free Medium 199 maintained at the level of the conjunctival epithelium. Rates of re-epithelialisation in response to addition of exogenous epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and transforming growth factor type $ß_1$ (TGF-$ß_1$) were assessed by image analysis.
Results. Corneal cultures could be maintained for upto 3 weeks without significant stromal oedema or keratocyte deterioration and with little loss of epithelial architecture. Following wounding the cornea re-epithelialised in a similar fashion to that observed in vivo i.e. a lag phase followed by migration/proliferation and the reformation of an intact multilayered epithelium. EGF accelerated, basic FGF had no effect on, and TGF-$ß_1$ inhibited the rate of corneal re-epithelialisation.
Conclusions. This simple organ culture model offers many of the advantages of animal models while overcoming many of the disadvantages of cell culture. In particular, it a) maintains the corneal architecture, b) allows interaction between the different cell types in the cornea i.e. keratocytes and epithelial cells, c) takes into account the role of limbal cells and centripetal migration, d) enables the specific effect of exogenous factors to be evaluated, and e) permits the use of human tissue.

CULTURED BOVINE CORNEAL EPITHELIAL CELLS IN A SMALL APPLIED ELECTRIC FIELD: EFFECTS OF EGF, bFGF AND TGF

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Purpose Certain growth factors, or an applied electric field(EF) induce directional migration of cultured corneal epithelial cells (CEC). Serum in culture medium dramatically lowers the threshold for electric field induced perpendicular reorientation and cathodal directed migration of cultured bovine epithelial cells. Both dissociated cells and cell sheets show enhanced responsiveness to EF in the presence of serum. We have studied the interaction of growth factors with EF in promoting directed cell migration.
Methods Serum free medium and media with EGF, bFGF and TGF were exchanged into primary cultures of bovine CEC immediately prior to field application. Serial photographs were taken and analyzed.
Results Cells in serum free media showed no directional migration.
EGF (epidermal growth factor), bFGF (fibroblast growth factor–basic) and TGF (transforming growth factor–beta 1) induced perpendicular reorientation and promoted dissociated cell and cell sheet migration toward the cathode. At a field strength of 150mV/mm, there was a significant increase in the proportion of cells realigning perpendicular to the EF. Additionally, in bFGF and TGF cell sheets expanded to cover a greater area, by extending membrane preferentially towards the cathode. The optimal concentrations of growth factors producing significant cathodal migration at 150mV/mm were, EGF 25ng/ml:4.8±0.8um/h, bFGF 100ng/ml:4.6±0.8um/h, TGF 1pg/ml: 5.0±1.0um/h. This represents a restoration of migration to about one third of the control migration rate in serum (13.3±2.6um/h). The growth factors also restored cathodal migration of epithelial sheets to nearly half of the control rate with serum (17±1.9um/h).
Conclusions Interactions of single growth factors with an applied EF promotes cell migration in vitro. Using combinations of these and other growth factors may prove useful in of clinical attempts to promote corneal wound healing. (supported by the Wellcome Trust)